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1) Gaffney et al.
"Enzyme linked immunoassay with monoclonal antibody for human interleukin-1-beta"
BIOTECHNIQUES
Vol. 5 (7)
p. 652-654
1987 please inlcude the coverpage with the month of publication,thanx

2) Di Giovine et al.
"Radio-immunoassay RIA of interleukin 1-beta IL1 in acute and chronic reheumatic diseases"
BR. J RHEUMATOL
Vol. 26 (suppl. 1)
p. 34
April 9-10, 1987

3) Censini et al.
"antibodies to human interleukin 1 beta"
LYMPHOKINE RES
Vol. 6 (1)
p. ?
Jan 11-15, 1987

OR 435.8.L93L95

thanx

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A PARTIALLY PURIFIED URINARY IL-1 INHIBITOR THAT INTERFERES WITH IL-1 BINDING. P. Seckinger, K. Williamson, J.-M. Dayer, Division of Immunology & Allergy, Hôpital cantonal universitaire, Geneva, and J.W. Lowenthal, H.R. Macdonald, Ludwig Institute for Cancer Research, Epalinges/Lausanne, Switzerland.

Urine from febrile patients was found to contain a novel inhibitor blocking IL-1 activity. Partial purification of this inhibitor involved ultrafiltration, 40 - 80% ammonium sulfate precipitation, ion exchange chromatography, affinity chromatography, and ACA54 gel filtration. Inhibitory activity eluted with an apparent molecular weight of 18 - 25 kD and blocked IL-1-dependent murine thymocyte proliferaton, PGE_2/collagenase production by human synovial cells and dermal fibroblasts, and fibroblast proliferation. The mechanism of action was investigated and the inhibitor shown to block, in a dose-dependent fashion, the specific binding of ^{125}I-IL-1 α to its high and low affinity receptors on the murine thymoma cell line EL-4. Stronger ^{125}I-IL-1 binding inhibition was observed when the cells were preincubated with the inhibitor and was largely irreversible at 4°C. Washing the cells after preincubation did not significantly decrease the capacity of the inhibitor to block ^{125}I-IL-1 binding. Several parameters - such as Western blotting with a polyclonal antibody to human IL-1 - indicate that the inhibitor is distinct from IL-1 or its fragments. The data provide the first evidence for a mechanism of action of an IL-1 inhibitor that acts *in vitro* by blocking IL-1 binding to its receptor.

1112

IDENTIFICATION OF AN IMMUNOREGULATORY DOMAIN OF HUMAN INTERLEUKIN 1 BETA

P. Ghiara, S. Censini, G. Volpini, L. Nencioni, L. Villa, M. Bartalini, R. Presentini, F. Perin, G. Antoni, A. Tagliabue and D. Boraschi

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In order to identify the minimal structure responsible for the immunostimulatory activity of IL-1, peptide fragments of human and murine IL-1 were synthesized on the basis of their computer-predicted exposure on the surface of the molecule. One of them, fragment 163-171 of human IL-1ß (hu IL-1ß), was found to exert biological activity. The biological properties of this active moiety of IL-1 make it particularly interesting for a possible clinical application. In fact, peptide 163-171 was able to mediate both "in vitro" and "in vivo" the immunostimulatory activities of IL-1 (T-cell activation, IL-2 induction, adjuvant effect) but it was apparently not involved in those untoward inflammatory reactions (eicosanoid synthesis, neutrophil activation, induction of acute phase proteins and fever) that represent major drawbacks for the therapeutic use of the entire IL-1 molecule in immunorestoration.

Thus, our study suggests that the multiple biological activities of IL-1 may be mediated by different moieties within the polypeptide chain and provide a new insight of lymphokine action at the molecular level.

1113

ANTIBODIES TO HUMAN INTERLEUKIN 1 BETA

S. Censini, P. Ghiara, G. Scapigliati, G. Volpini, G. Antoni, F. Perin, R. Presentini, M.C. Casagli, D. Nucci, M. Bigio, R. Rossi, A. Tagliabue and D. Boraschi

Sclavo Research Center, Siena, Italy

In order to develop specific antibodies (Abs) to human IL-1 (hu IL-1), a computer-aided analysis of possible antigenic sites was performed on the cDNA-deduced sequence of hu IL-1ß. Two of these possible sites were chosen (fragments 163-171 and 189-197), synthesized and coupled to keyhole limpet haemocyanin (KLH). Peptide-KLH conjugates were utilized for immunizing rabbits and mice and specific polyclonal (PAbs) and monoclonal (MAbs) antibodies were obtained. MAbs and PAbs to both peptides were able to recognize hu IL-1ß in RIA and ELISA in a dose-dependent and domain-specific fashion. Furthermore, PAbs were able to recognize recombinant hu IL-1ß (hu rIL-1ß) in western blot analysis of crude concentrated supernatants.

In another series of experiments, a MAb against hu rIL-1ß was obtained and found to react both in immunoblottings of the purified immunization antigen and in indirect immunofluorescence on paraformaldehyde-fixed and unpermeabilized U937 human hystiocytic lymphoma cells.

Thus these reagents may prove to be useful tools in the detection of IL-1 in biological fluids and on cells.

1114

INDUCTION OF IL-1 RELEASE BY NATURAL AND SYNTHETIC LIPID A AND LIPID A PART STRUCTURES

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The lipid A portion of lipopolysaccharides (LPS) is known to be responsible for the endotoxic activities of LPS. The availability of synthetic lipid A and part structures enabled us to study their properties to induce IL-1 release from human peripheral blood monocytes. The preparations used include bacterial and synthetic (compound 506) Escherichia coli hexaacyl lipid A, the 4'-dephospho (505) and 1-dephospho (504) part structures, as well as synthetic tetraacyl precursor Ia (406), its 4'-dephospho (405) and 1-dephospho (404) part structure. Furthermore, a pentaacyl derivative of lipid A (precursor Ib; compound LA 20) and its isomer (compound LA 21) were tested. IL-1 was measured as comitogenic activity in a murine thymocyte proliferation assay and as growth promoting activity for human fibroblasts.

Synthetic lipid A (506) stimulated IL-1 production to a degree comparable to bacterial E.coli lipid A and Re mutant LPS. Compound 505 was of significantly higher activity (factor of 10), while compound 504 was less active than compound 506. Synthetic precursor Ia (406) and its monodephospho derivatives failed to stimulate IL-1 production. Compounds 20 and 21 were only weakly effective in inducing IL-1 release. The release of IL-1 activity induced by synthetic compounds followed the kinetics known for IL-1 induction with LPS. The released activity was partially heat labile and was inhibited by antiserum directed against human leukocytic pyrogen (kindly provided by Dr. Ch.A. Dinarello). No IL-2 activity was detectable in supernatants of lipid A stimulated cells.

We conclude that lipid A and part structures thereof are able to stimulate the release of IL-1 from human monocytes, as known for LPS. We also conclude that hexaacylation of lipid A is required for effective IL-1 release.

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